

**Summary Report on the Effects of Assail 70 WP Straight and in Combination with Procure 50 WS on Honey Bees (*Apis mellifera* L.)**

PMRA Submission Number N/A

EPA MRID Number 459325-04

**0Data Requirement:**

PMRA Data Code: N/A

EPA DP Barcode: Not Provided

OECD Data Point: None

EPA Guideline: None

D 355625

**Test Material:** Assail 70 WP**Purity:** 73.89%**Common name:** Acetamiprid**Chemical name:** IUPAC: Not reported

CAS name: Not reported

CAS No.: 135410-20-7

Synonyms: EXP61842A

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**{EPA/OECD/PMRA}****Date:** 08/19/2008**Reference/Submission No.:** {.....}**Company Code** {.....} [For PMRA]**Active Code** {.....} [For PMRA]**Use Site Category:** {.....} [For PMRA]**EPA PC Code** 099050**Date Evaluation Completed:** {dd-mm-yyyy}

**Citation:** Schur, Andrea. 2002. A Semi-Field Study on the Effects on Honey Bees (*Apis mellifera* L.) of ASSAIL 70 WP (EXP61842A, Acetamiprid 70%) Straight and in Combination with the Fungicide PROCURE 50 WS (Triflumizole 50%). Unpublished study performed by Arbeitsgemeinschaft, GAB Biotechnologie GmbH, Niefern-Oschelbronn, Germany. Laboratory report number 20011239/S1-BZEU. Study sponsored by Nippon Soda Co., Ltd., Chiyoda-ku, Tokyo, Japan. Study completed November 5, 2002.

**SUMMARY:**

The semi-field study was carried out following the EPPO Guideline No. 170: Guideline on methods for evaluating the side-effects of plant protection products on honey bees (EPPO, 1992). Colonies were treated with five different treatment applications. The study was conducted in a field of flowering *Phacelia tanacetifolia* Benth where tunnel tents were placed over colonies. There were three acetamiprid treatments in which EXP61842A was applied at a rate equivalent to 168 g ai/ha in 300 L water/ha. In one group (T1) EXP61842A was applied straight on the flowering crop. A second group (T2) comprised a tank-mixture of EXP61842A and the fungicide PROCURE 50WS (containing the active ingredient triflumizole; 561 g ai/ha) in 300 L water/ha. The third group (T3) the fungicide (561 g ai/ha in 300 L water/ha) was applied one day before treatment with EXP61842A, which was applied to the treated plots. A fourth group was treated with tap water to serve as a negative control and a fifth group was treated with Dafene 40L at a concentration of 680 g product/ha in 300 L water/ha to serve as a toxic standard.



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Mortality, behavior, foraging activity, condition of the colonies and development of the bee brood was assessed before and after treatment. The purpose of this test was to evaluate potential worst-case exposure conditions as the applications were conducted in tunnels at periods of bee foraging activity. Exposure in this type of semi-field study is more intense than that experienced by free-living bees from standard apiaries. In this type of semi-field test, foraging is restricted to the treated crop inside the tunnel and exposure may compromise all main exposure routes (contact by overspray and tarsal exposure to fresh and dry residues in vegetation and oral exposure by ingestion of contaminated pollen or nectar).

Application of a tank mixture of acetamiprid and the fungicide (T2) caused an increase in total mortality one day after application. Applications with acetamiprid straight on the flowering crop (T1) and application of the tank mixture of acetamiprid and the fungicide (T2) caused a reduction in flight activity recorded approximately 1 hour after treatment on the day after application. Acetamiprid treatment (T1 and T3) appeared to impact brood development, with missing brood stages (egg and/or larval) during earlier portions of the assessment; however, all colonies recovered once hives were transferred out of the tents. Although mortality was somewhat higher in the treatment groups, bee behavior was not adversely impacted by treatment.

This study is classified 'supplemental', as it contains information useful for risk assessment purposes, but is not conducted under any Agency guideline.

## **METHODOLOGY:**

### **Test Materials:**

The primary test material was the insecticide Assail 70WP (formulation containing the active ingredient acetamiprid, 73.89%) which was described as a beige powder and stored under ambient conditions. The other test material was the fungicide Procure 50WS (formulation containing the active ingredient triflumizole, 51.9%) which was described as a solid and stored under cool and dry conditions. The toxic standard was the insecticide Dafene 40L (formulation containing the active ingredient dimethoate, 400 g/L) which was described as a beige liquid and stored under ambient conditions.

### **Test Organism:**

The honey bee (*Apis mellifera* L; Hymenoptera, Apidae) was used as the test organism. The honey bee is an important beneficial insect due to its pollination activity in fruit, berry and seed growing. Due to the specific use of honey bees in the crops pollinated (migratory beekeeping) they are an important productive factor. Additionally, they contribute to the preservation of a multitude of wild flowering plants because of their pollination activity.

### **Test Site:**

The field where the study was conducted was located in the province of Valencia in Spain. The crop used was *Phacelia tanacetifolia* which is recommended in guideline EPPO 170 for tunnel testing. The field used was approximately 1000 m<sup>2</sup>. Ten (10) tunnel tents were set up in the field with two tunnels for each of the five treatment groups. The 10 tunnel tents were set-up in the field soon before moving the hives to the experimental field.

*Phacelia* covered about 50 m<sup>2</sup> per tent. The size of each tunnel (covered plot) was 12 m long, 5 m wide and 3.5 m high in the center. Once the full flowering stage had been reached, paths were made into each tunnel by removing the plants and smoothing the ground. The paths were then covered with linen sheets and the tent frames were covered with light plastic gauze. On the same day, one small colony was introduced into each test. A bucket half-filled with water was introduced as well, with scraps of polystyrene on the surface to prevent the bees from drowning.

### **Experimental Bee Colonies:**

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Small healthy colonies with four combs were used for the test. All nuclei were produced at the same time and each colony contained approximately 1000 adult worker bees. Additionally, the following criteria for each nuclei were met:

- at least two brood combs present containing eggs, larvae and capped cells (except one colony in the test group T1 and T2)
- at least one honey and pollen comb present
- bees were free of symptoms of *Nosema* disease

To register dead bees which were carried out of the hives, wooden bee traps (38 x 38 x 22 cm) with gauze on the bottom and on 50% of the top were attached to the entrance of the nucleus. The hives were placed into the tents 3 days before the planned application of the test material to allow the bees to familiarize with the environment and to lower the mortality, which is usually increased due to stress from transport. After the end of the exposure period, the hives were removed from tents and transferred to an area where no pesticides were used (mountains).

## Application:

Test solutions were prepared shortly before each application. The applications were carried out up to midday with a plot sprayer that emulates a commercial application. During all applications, the *Phacelia* crop was in full bloom, bees were actively foraging and the wind speed was below 2 m/sec. After the treatment of each plot, the volume applied was verified by measuring the remaining spray solution. The accepted spray tolerance was  $\pm 10\%$  for the whole plot. The actual applied amounts of spray solution ranged from 1.40 to 1.65 L/ha

## Assessments:

Mortality was assessed by observation of the wooden bee traps to determine the number of dead bees carried out of the hive and by observation of the linen sheets spread around the *Phacelia* plants. Mortality was assessed once a day beginning three days prior to application, immediately before and after application, and daily for seven days after application.

Flight intensity was determined by counting the number of bees that were both foraging on flowering crops and flying immediately over the crop within a 1 m<sup>2</sup> section during a one-minute observation period. The square observed was chosen at random. Assessments were made once a day beginning three days prior to application, immediately before application, 1, 2, and 5 hours after application, and daily for seven days after application.

The condition of the colonies was assessed 2 days prior to moving the colonies to the tents, at the end of the exposure in the tunnels (8 days after application), and 1 month after moving the hives to an untreated area. The condition and development of the bee brood were assessed using the following parameters:

- strength of colony (number of combs covered with bees)
- presence of a healthy queen (presence of eggs, presence of queen cells)
- estimate of the pollen storage area and area with nectar
- estimate of the area containing eggs, larvae and capped cells

The amount of eggs, larvae and capped brood was given in percent of total brood population for each type of brood.

## Evaluation of Results:

The influence of the test substance was evaluated by comparing the bees in the treatment tents to the control bees and toxic standard treatment group with the following observations:

- Mortality at the edge of the treated area and in the bee traps
- Foraging activity (number of forager bees/minute/m<sup>2</sup> flowering *Phacelia* crop)
- Behavior of the bees on the crop and around the hive)
- Development of the bee brood

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## **RESULTS:**

### **Mortality:**

For the three days prior to application mean mortality (bee trap + linen sheets along edge of treated area) from both tents averaged 35.3 in the control, 65.0 in T1 (Assail 70WP only), 55.9 in T2 (Assail 70WP + Procure 50WS), 51.8 in T3 (Procure 50WS followed by Assail 70WP), and 59.8 in the toxic standard treatment. Seven days after application, mean mortality was 8.8 bees/day in the control, 20.6 bees/day in T1, 28.9 bee/day in T2, 18.2 bees/day in T3, and 218.5 bees/day in the toxic standard treatment.

**Table 1. Mortality of *Apis mellifera* before and after exposure of Assail 70 WP and Procure 50 WS**

Treatment Group	Pre-Application			Post-Application		
	Mean Bee Trap	Mean Linen	Mean/Tent and Day	Mean Bee Trap	Mean Linen	Mean/Tent and Day
Control	7.1	28.1	35.3	1.1	7.8	8.8
T1	4.5	60.5	65.0	2.4	18.2	20.6
T2	6.5	49.4	55.9	9.6	19.3	28.9
T3	4.5	47.3	51.8	3.2	15.0	18.2
Toxic Standard	4.6	55.1	59.8	127.3	91.2	218.5

The mean bee trap and mean linen values represent the mean of dead bees observed each day between the two tents. The Mean/Tent and Day value is the overall mean value of dead bees observed in the bee trap and on the linen from both tents over the entire duration.

### **Flight Intensity and Behavior:**

For the three days prior to application, flight intensity between the two tents averaged 10.1 number of bees/minute/m<sup>2</sup> in the control group, 10.4 number of bees/minute/m<sup>2</sup> in T1, 10.9 in number of bees/minute/m<sup>2</sup> T2, 9.4 number of bees/minute/m<sup>2</sup> in T3, and 11.0 number of bees/minute/m<sup>2</sup> in the toxic standard group. By test termination (7 days after application) flight intensity between the two tents averaged 21.5 number of bees/minute/m<sup>2</sup> in the control group, 20.1 number of bees/minute/m<sup>2</sup> in T1, 20.5 number of bees/minute/m<sup>2</sup> in T2, 21.7 number of bees/minute/m<sup>2</sup> in T3, and 1.5 number of bees/minute/m<sup>2</sup> in the toxic standard group.

All bees in the control and T3 tents appeared normal on the day of application. Approximately 1 hour after application, reduced flight intensity was observed in the T1 and T2 tents; however, all bees in the T1 and T2 tents appeared normal with regular flight intensity by the next observation period one hour later. A repellent effect was noticed in the toxic standard group 1, 2 and 5 hours after application of the test material.

**Table 2. Flight intensity of *Apis mellifera* before and after exposure of Assail 70 WP and Procure 50 WS**

Treatment Group	Pre-Application (bees/minute/m <sup>2</sup> )			Post-Application (bees/minute/m <sup>2</sup> )		
	Tent 1	Tent 2	Mean	Tent 1	Tent 2	Mean
Control	10.0	10.3	10.1	20.1	22.8	21.5
T1	10.8	10.0	10.4	19.4	20.9	20.1
T2	10.8	11.0	10.9	21.5	19.5	20.5
T3	9.0	9.8	9.4	20.9	22.6	21.7
Toxic Standard	11.3	10.8	11.0	1.8	1.2	1.5

### **Brood Development:**

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Prior to application of the test material, the strength and number of combs covered with brood were similar at all treatment levels, control and toxic standard tents and all developmental stages were observed. In all treatment groups, including the control and toxic standard tents, the strength and number of combs covered with brood either remained stable or increased until the first observation after application (8 days later) and one month after moving the hives to an untreated area.

The first assessment after application of the test material indicated that the egg and larval stage was missing in the colony of tent 1 in the test group T1. In the colonies of the test group T3, no larval stage was recorded. The other colonies of the test group T2, the control and toxic standard showed all brood stages.

At the last assessment, one month after transferring the colonies to an untreated area, the colonies of the test substance treatments (T1, T2 and T3) the control and the toxic standard treatment showed all brood stages at the end of the test, which confirms that the bee hives were in good condition and the colonies with a lack of eggs and/or larvae in the test group T1 and T3 were able to recover from the first brood assessment after the application to the last brood assessment.

Assessment	Brood Development					
	Pre-Application		8 Days After Application		1 Month After Transfer	
	Tent 1	Tent 2	Tent 1	Tent 2	Tent 1	Tent 2
<b>Control</b>						
Strength <sup>a</sup>	4.00	4.00	4.50	4.50	4.50	4.50
# of Combs	2.00	2.00	2.00	2.00	4.00	3.00
Egg <sup>b</sup>	20.00	10.00	25.00	22.50	6.25	20.00
Larval <sup>c</sup>	10.00	12.50	7.50	2.50	8.75	6.67
Capped <sup>d</sup>	22.50	42.50	27.50	17.50	25.00	16.67
<b>T1 (Assail 70 WP; 168 g ai/ha in 300 L water/ha)</b>						
Strength <sup>a</sup>	4.00	4.00	4.00	4.50	4.50	4.50
# of Combs	2.00	2.00	2.00	2.00	3.00	4.00
Egg <sup>b</sup>	20.00	17.50	0.00	7.50	11.67	12.50
Larval <sup>c</sup>	17.50	0.00	0.00	7.50	6.67	13.75
Capped <sup>d</sup>	32.50	15.00	22.50	22.50	33.33	22.50
<b>T2 (Assail 70 WP 168 g ai/ha + Procure 50 WS 561 g ai/ha in 300 L water/ha)</b>						
Strength <sup>a</sup>	4.00	4.00	4.00	4.00	4.50	4.50
# of Combs	2.00	2.00	3.00	2.00	3.00	4.00
Egg <sup>b</sup>	30.00	37.50	20.00	7.50	11.67	12.50
Larval <sup>c</sup>	7.50	0.00	6.67	7.50	16.67	5.00
Capped <sup>d</sup>	30.00	12.50	20.00	30.00	30.00	30.00
<b>T3 (Procure 50 WS 561 g ai/ha on Day -1, Assail 70 WP 168 g ai/ha on Day 0; 300 L water/ha)</b>						
Strength <sup>a</sup>	4.00	4.00	4.00	4.00	4.50	4.50
# of Combs	2.00	2.00	2.00	2.00	3.00	4.00
Egg <sup>b</sup>	15.00	5.00	27.50	22.50	15.00	10.00
Larval <sup>c</sup>	20.00	12.50	0.00	0.00	16.67	13.75
Capped <sup>d</sup>	40.00	47.50	17.50	22.50	25.00	26.25
<b>Toxic Standard (Dafene 40L 680 g product/ha in 300 L water/ha)</b>						
Strength <sup>a</sup>	4.00	4.00	4.00	4.00	4.00	4.00
# of Combs	2.00	2.00	2.00	2.00	3.00	3.00
Egg <sup>b</sup>	10.00	15.00	25.00	10.00	15.00	11.67
Larval <sup>c</sup>	15.00	15.00	7.50	2.50	10.00	6.67
Capped <sup>d</sup>	50.00	47.50	20.00	7.50	31.67	11.67

<sup>a</sup> Strength was determined by the number of combs covered with bees

<sup>b</sup> Average amount (%) of egg stage

<sup>c</sup> Average amount (%) of larval stage

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<sup>d</sup> Average amount (%) of capped stage

## **CONCLUSIONS:**

### **Mortality:**

The application of Assail 70 WP only (T1) and the application of Assail 70 WP one day following application of Procure 50 WS (T3) caused an observed, though not statistically significant, increase in the number of dead bees. In T2, a tank-mixture of the two test materials caused an increase in the total mortality (dead bee trap and linen sheets) immediately after application with 74.5 dead bees/tent and 75.5 dead bees/tent one day after application compared to the pre-application mortality of 55.9 dead bees/tent/day. The application of the toxic standard caused an obvious increase in mortality (339.5 dead bees/tent/day following application).

### **Flight Intensity and Behavior:**

The level of flight activity, measured as the number of foraging bees in one minute over a one-square meter area, was similar during the pre-application period. The applications in the T1 and T2 groups did cause a reduction in flight activity restricted to the assessment carried out approximately 1 hour after application. No decrease in flight activity was observed on the day of application in the T3 group. The toxic standard caused a clear reduction in flight activity during the entire post-application period. No difference in the average flight activity was observed in the three treatment groups relative to the control flight activity throughout the post-application period.

No abnormal difference in behavior of the bees was observed between the test substance treatments and the control at any time during the exposure periods.

### **Brood Development:**

No adverse effect on brood development could be observed after the treatments in the test substance group T2. In the colonies of tent 1 in test group T1, the egg and larval stages were missing and in the colonies of the test group T3, no larval stage was recorded at the first assessment after treatment. This lack of brood stages in the colonies was attributed to the loss of queens, which possibly resulted from damage to the queens during the first assessment or an irritation of the worker bees because of the condition in tents with the reaction to terminate the queen in those colonies. At the last brood assessment approximately one month after moving the hives out of the tents, almost every colony increased the number of combs covered with brood and the strength of the colonies and all brood stages in the colonies of the different treatments were available.

## **References:**

EPPO. 1992. Guideline on test methods for evaluating the side-effects of plant protection products on honey bees- EPPO Bulletin 22, 203-216.